



Comparison of fungal communities associated with spruce seedling roots and bryophyte carpets on logs in an old-growth subalpine coniferous forest in Japan



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ABSTRACT

Fungal communities associated with plant tissues were compared between two bryophyte species dominating decaying logs (*Scapania bolanderi* and *Pleurozium schreberi*), and roots of spruce seedlings growing on the bryophytes and in the ground soil, to evaluate the contribution of fungal communities to seedling regeneration. Using high-throughput DNA sequencing, a total of 1233 fungal operational taxonomic units (OTUs) were detected. Saprotrophic Ascomycota were dominant in bryophytes, whereas ectomycorrhizal (ECM) Basidiomycota were dominant in spruce roots. Fungal communities were significantly different between the two bryophyte species. In addition, fungal communities of spruce seedlings were significantly affected by the substrates on which they were growing. Some ECM fungi were detected from both of the bryophytes and the spruce seedlings growing on them; however, the dominant OTU identities differed between the two bryophyte systems. The possible effects of functional differences between dominant fungal OTUs on spruce seedling regeneration are discussed.

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1. Introduction

Growing evidence has demonstrated that belowground microbial communities have an essential role in the dynamics of aboveground vegetation (Bardgett and Wardle, 2010). For example, plant root pathogenic fungi cause diseases of plants and reduce their growth and survival (Gilbert, 2002). In contrast, mutualistic fungi, such as mycorrhizal and root endophytic fungi, improve nutrient and water uptake of plants, reduce the attack of pathogens on roots and increase plant performance (Smith and Read, 2008). The effects of belowground fungi on plants are particularly important during the establishment stage of fragile juvenile seedlings. In forested ecosystems, understory microhabitats for tree seedlings are diverse, e.g. downed logs, stumps, mounds, pits, rocks, bryophyte carpets, ground covered with a thick litter layer, and bare soil. It is crucial to study the composition of fungal communities in a variety of microhabitats in forests to understand the process of seedling establishment (Tedersoo et al., 2008).

In boreal and subalpine coniferous forests, downed logs are important microhabitats for seedling regeneration (Harmon et al., 1986). Seedlings colonizing decayed logs can avoid soil pathogens causing damping-off diseases (O'Hanlon-Manners and Kotanen, 2004) and can establish symbiotic associations with stable mycorrhizal communities (Tedersoo et al., 2008). In addition, a conspicuous feature of logs in boreal and subalpine forests is the high coverage by bryophyte carpets (Ódor and van Hees, 2004; Bardgett and Wardle, 2010), which is critical for seedling establishment (Nakamura, 1992; Hornberg et al., 1997; Iijima and Shibuya, 2010). Bryophyte carpets can promote seedling colonization and survival by trapping seeds (Harmon, 1989), protecting seeds from herbivores (van Tooren, 1988), retaining water and nutrients (Takahashi et al., 2000; Sand-Jensen and Hammer, 2012) and having an anti-freezing effect (Jeschke and Kiehl, 2008). In contrast, bryophytes are also reported to suppress seedling regeneration by their thick dry carpets (Delach and Kimmerer, 2002), reduced light conditions (Harmon and Franklin, 1989; Nakamura, 1992; Iijima and Shibuya, 2010), competition for nutrients (Zackrisson et al., 1998), reduction in temperature fluctuations (Soudzilovskaia et al., 2011) and allelopathic effects (Michel et al., 2011) preventing seed germination and/or seedling growth and

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survival.

Recent studies demonstrated the importance of fungi in bryophyte carpets for seedling colonization. Hyphal abundance of symbiotic mycorrhizal fungi was higher in bryophyte carpets than in bare soil (Cappellazzi et al., 2007), and bryophyte carpets reduced the risk of plant pathogenic fungal infection of tree seeds and improved germination rate (Wang et al., 2016). In contrast, direct translocation of nutrients from bryophytes to ericaceous trees via ericoid mycorrhizal fungi reduce nutrient release from senescent bryophyte tissues, and thus can reduce nutrient acquisition and growth of conifer seedlings that have no association with ericoid mycorrhizal fungi (Zackrisson et al., 1997). The relative importance of these positive and negative effects of bryophyte carpets via fungal communities has been poorly addressed. Since the influence of fungi on seedling performance can vary between fungal species, it is important to examine species identity and functional guild composition of fungi within bryophyte carpets to understand their effects on conifer seedling establishment on downed logs.

Recently, a strong, species-specific effect was demonstrated of bryophytes on spruce (*Picea jezoensis* var. *hondoensis*) seedling density on decayed logs in an old-growth subalpine coniferous forest in Japan (Ando et al., 2017). Spruce seedling density was significantly higher in carpets of a liverwort *Scapania bolanderi* than in a moss *Pleurozium schreberi*, although both bryophyte species dominated on the logs. Although the thickness of these carpets varies significantly between these two bryophyte species (Ando et al., 2017), which has traditionally been thought to be the primary factor affecting seedling performance in bryophyte carpets (Iijima and Shibuya, 2010), carpet thickness is not likely to significantly affect spruce seedling density in this system (Ando et al., 2017). In the present study, we focused on fungal communities associated with bryophyte carpets to reveal their possible effects on spruce seedling regeneration on decayed logs using amplicon sequencing. Given that liverworts and mosses (herein 'bryophytes') have distinctive evolutionary and symbiotic histories with fungi, communities of their fungal associates could be different between bryophyte species and have different effects on spruce seedlings. If the mutualistic fungi are dominant in *S. bolanderi* carpets and are also detected from spruce seedling roots, those fungi could contribute to the higher density of seedlings on *S. bolanderi* carpets. Similarly, this could arise through lower abundance of plant pathogenic fungi in *S. bolanderi* compared with *P. schreberi* carpets. We had several working hypotheses: (1) bryophyte-associated fungal communities are significantly different between the two dominant bryophyte species coexisting on logs; (2) seedling root-associated fungal communities differ among the seedlings regenerating on different substrates; (3) ectomycorrhizal or endophytic fungi beneficial for spruce seedlings are more dominant in the *S. bolanderi* than in the *P. schreberi* system; (4) plant pathogenic fungi, which could increase seedling mortality, are less dominant in the *S. bolanderi* than in the *P. schreberi* system and (5) some root-associated symbiotic (mycorrhizal and endophytic) fungi are shared with the bryophytes. We also analysed root-associated fungal communities in spruce seedlings collected from the ground soil for comparison.

2. Materials and methods

2.1. Study site

The present study was conducted in an old-growth subalpine coniferous forest (2050 m a.s.l., 35°56'N, 137°28'E) on a north-facing slope of Mt. Ontake (3067m a.s.l. at the peak) in central Honshu, Japan. The annual precipitation from 1984 to 2014

recorded near the study site ranged from 1760 to 5349 mm, with an average of 2385 mm. The mean annual temperature is 3–4 °C. Snow covers the forest floor from mid-November or early December to late May or early June. The primary natural disturbances are large-scale canopy disturbances caused by typhoons, but no anthropogenic disturbances have been recorded in the study site. A 1 ha permanent plot was established in the study area in 2001. Within this plot, vegetation and regeneration patterns of dominant tree species (Mori and Takeda, 2004; Mori et al., 2004; Ando et al., 2017) and decay patterns of logs (Fukasawa et al., 2014) have been described. Dominant canopy tree species (basal area) are *Picea jezoensis* var. *hondoensis* (16.51 m² ha⁻¹), *Abies veitchii* (6.95 m² ha⁻¹), *A. mariesii* (9.29 m² ha⁻¹), *Tsuga diversifolia* (6.05 m² ha⁻¹) and *Betula ermanii* (6.94 m² ha⁻¹). Understorey vegetation in the plot consists of herbs and bryophytes, with a few *Sasa* species, which is the dwarf bamboo that dominates the understorey of many Japanese forests. The forest floor is covered with 13.9% downed logs and stumps, 4.9% uprooting mounds, 1.8% rocks and 79.4% ground (Mori et al., 2004). The majority of the surface of downed logs and stumps is covered with bryophyte carpets dominated by *S. bolanderi* (liverwort) and *P. schreberi* (moss) (Ando et al., 2017).

2.2. Sample collection

In June 2014, living bryophyte carpets (each approximately 15 cm × 15 cm) and spruce seedlings were sampled from the study site. Bryophyte carpets were sampled from the two dominant species on the logs (Ando et al., 2017), *S. bolanderi* (17 carpets) and *P. schreberi* (11 carpets). Spruce seedlings (current-year, 1-year-old and over 2-year-old, less than 5 cm in height) were sampled from *S. bolanderi* carpets (17 seedlings), *P. schreberi* carpets (11 seedlings) and ground near the sampled logs (7 seedlings). All samples were separately packed in nylon bags, stored at –20 °C until further processing.

Five pieces of bryophyte stems, 5 mm in length for each (3 pieces from green part, 2 pieces from senescent brown part) were collected from each bryophyte carpet and were pooled to make a bryophyte sample. Eight root tips (approximately 20–100% of all root tips) were randomly collected from root systems of each seedling regardless of ectomycorrhizal colonization, and were pooled to make a root sample. All soil and wood particles were carefully removed from bryophyte stems and seedling roots using forceps under the binocular microscope, and samples were washed with sterile distilled water using hypersonic vibration to remove fine soil particles. Further, samples were put into a 2-ml tube with 70% ethanol and 1-mm zirconium beads and then shaken 15 times per second for 2 min using a Mixer Mill MM300 (Retch, Germany) to clean the surface (Toju et al., 2013). Finally, samples were washed with sterilized water again and then stored at –30 °C until DNA extraction.

As DNA-barcoding-based analysis does not provide any information on the nature of symbiosis between plants and their fungal associates, we treated the observed association between fungi and plants (bryophytes and seedlings) in a broad sense of symbiosis (Thompson, 2005). Although taxonomic information may help to infer potential ecological roles of each fungus, it is important to acknowledge that fungi detected through high-throughput sequencing can be not only mutualistic, but also commensalistic or antagonistic to their host plants. (Toju et al., 2014).

2.3. DNA extraction and sample preparation for rDNA ITS sequencing

Fungal ITS amplicons were sequenced from bryophyte and

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